Indian J. Phys. **78B**(1), 103-105 (2004)

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AN INTERNATIONAL JOURNAL IJP B

Elemental analysis of anti-diabetic medicinal plants using energy dispersive X-ray fluorescence technique

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Received 9 September 2003, accepted 6 November 2003

Abstract : Aquantitative energy dispersive X-ray flourescence method, based on molybdenum target, applicable to plant samples, is described in this work. The results obtained from the elemental analysis of leaves of eleven different plants commonly used for curing diabetes mellitus in eastern India are also presented. The elements K, Ca, Fe, Cr, Mn, Cu, Zn, Rb, Sr and Pb are identified and quantified simultaneously in these leave samples. Concentration of these elements shows variation in their proportions. Among these elements, potassium and calcium have been estimated in percentage level whereas other elements are quantified in ppm level

Keywords : Diabetes mellitus, energy dispersive X-ray fluorescence, medicinal plants, trace elements

PACS Nos. : 87.15.Mi, 82.80 Ej, 78 70.En

In modern world, popularity of various medicinal plants and plant therapies in treating various diseases is increasing. In many developing countries, people are used to adopt many unconventional therapies including herbal medicines [1]. However, in India, medicinal plants are the major form of medicines being utilized in crude form or powdered form by many tribal people [2]. These therapies are often perceived as being natural and therefore free from industrial chemical inputs with possible low toxic effect to human beings. Their potential pharmacological action and affordability are other advantages in their prevailing use. In fact, medicinal plants have a greater tendency towards inorganic micronutrients or trace elements from soil, since they have high concentration of inorganic elements compared to common non-medicinal plants [3]. Though it is well known that certain trace elements are essential for the healthy growth of plants, the uptake of some nonessential elements by these plants can also enhance the medicinal property in specific cases [4]. So the quantitative estimation of various minor and trace element concentration are important for determining the effectiveness of medicinal plants. Diabetes mellitus is the world's largest chronic metabolic disease with presently over 150 million diabetic patients [5]. It is estimated that by the year 2025, the world will have more than 300 millions people with this endocrine disease, being India ranked top with around 57 millions [6]. Though various anti-diabetic drugs are availabe commercially for the management and control of diabetes mellitus, these are expensive and in certain specific cases, their efficacy decreases after prolonged use [7]. Another advantage of using anti-diabetic agents with plant origin is that it can control diabetes without any side effects. It is also reported that various elements present in plant leaves have either direct or indirect role in control and management of blood sugar level. Potassium, calcium and trace amount of chromium are responsible for the secretion of insulin from β-cells of islets of Langerhans. Zinc complex is the storehouse of insulin secreted from pancreas [8], which plays an important role in normal glucose metabolism. Chromium has important role along with zinc, calcium and manganese in glucose tolerance factor (GTF), which

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decreases the blood glucose level by utilizing insulin [9]. Furthermore, deficiency of chormium can cause impaired action of GTF and hence can cause diabetes mellitus [10]. In optimal insulin secretion, normal potassium concentration is required [11]. Potassium also helps in protein and carbohydrate metabolism of the body and its deficiency causes diabetic acidosis [12]. Subnormal zinc level can cause several liver diseases and its deficiency can cause diabetic symptoms like hypsomia, hypogeusia or even coma [13]. Apart from this, for maintaining healthy metabolism [14] and control of body wieght, Fe, Ca and Zn are necessary. Furthermore, the WHO expert committee [15] has recommended the through study of medicinal plants dealing with diabetes mellitus. In India medicinal plants have mostly been studied for their organic content and little are known about their trace element distribution, and so, using non-destructive technique like EDXRF spectroscopy. The present preliminary study was undertaken aiming at verifying the usefulness of energy dispersive X-ray fluorescence technique for quantifying the trace elements, and also to observe, if possible, the degree of distribution of these elements.

Fresh leaf samples of different plants were collected from various places of Bhubaneswar (Orissa). The leaves were washed with distilled water, dried in an oven at about 80°C overnight and subsequently powdered by using agate mortar. Fine powdered samples were mixed with pure cellulose in the ratio 1 : 1 by mass. The mixture was thoroughly ground, homogenized and thick targets were prepared. In a similar manner, thick targets of certified reference materials (CRMs) of apple leaves (NIST-1515), orchid leaves (NIST-1571), tomato leaves (NIST-1573) from National Institute of Standards and Technology (NIST, USA) were prepared and irradiated for quantification and verification of the results. The EDXRF set up used for the present study incorporates a low power (50 Watt) air-cooled, tungsten anode X-ray tube as an excitation source with triaxial geometry [16]. The X-rays from the tube which was operated at 35 kV with 0.6 mA current, were allowed to fall on a molybdenum secondary exciter [17]. Then sample pellets were irradiated with the characteristic K X-rays from the secondary exciter. The advantage of employing secondary exciter is to prevent high background and hence lower detection limit in the fluorescence spectra. The detection of the characteristic X-rays of the elements present in all the samples was carried out using a Si (Li) detector, cooled with liquid nitrogen, with a resolution of 180 eV at 5.9 keV [18]. A amplifier processed the signals from detector and spectra were recorded by using a PC-based multi-channel analyzer (MCA). The photo-peak areas in each spectrum were evaluated using the computer program AXIL Prior to irradiation of the samples, pellets of NIST CRMs were irradiated for calibration.

The scientific names, common names and local names of medicinal plants selected for the present study are provided in Table 1. The EDXRF spectrum obtained from the *Tulsi* leave sample is presented in Figure 1, which indicates the attendance of trace elements like Cr, Mn, Fe,

Table 1. Scientific name, common name and local name of the anti-diabetic medicianl plants.

Scientific name	Common name	Local name (in *Oriya*)
Aegle marmelos	Wood-apple	Bella
Azadirachata indica	Margosa	Nimba
Eugenia jambolana	Black berry	Jamun
Moringa oleifera	Moringa	Sajana
Momordica charantia	Bitter gourd	Karela
Murraya koenigii	Curry leaf tree	Bhursunga
Ocimum sanctum	Sacred basil	Tulasi
Vinca rosea	Vinca	Sadabihari
Michelia champaka	Champak	Swarna champa
Albizzia lebbeck	Rain tree	Sirisa
Gymnema sylverster	Periclopa of the woods	Gudmari

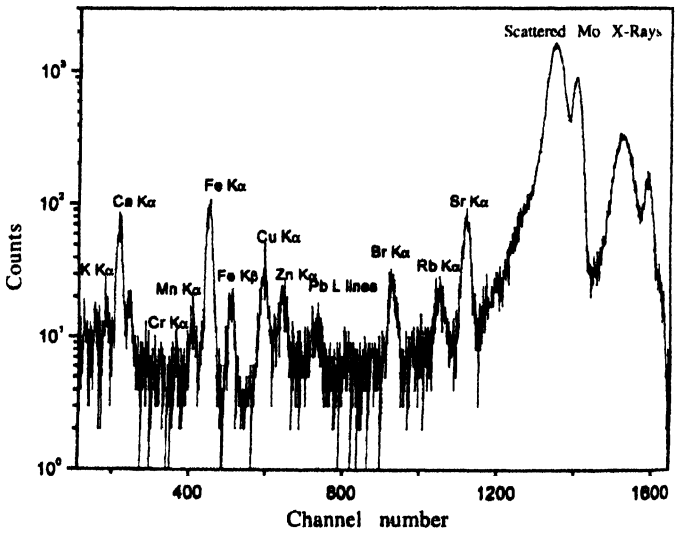

Figure 1. EDXRF spectrum of a *Tulasi* leave sample.

Sr, Pb along with major elements potassium and calcium. The concentration of different trace elements along with K and Ca obtained from the analysis of leave samples are provided in Table 2. Each value represents the mean of two independent measurements. *Tusli* with numerous medicinal properties [19,20] are found to contain higher amounts of Mn, Fe and Cu. *Karela* leaves used for

Table 2. Elemental concentrations in anti-diabetic medicinal plant leaves using EDXRF.

Elements*	Bella	Bhursunga	Sajana	Jamun	Nimba	Sadabihari	Sirisa	Swarna champa	Tulsi	Gudmari	Karela
K(%)	0.9 ± 0.1	1.6 ± 0.2	2.1 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	3.2 ± 0.1	3.9 ± 0.2
Ca(%)	0.9 ± 0.1	5.6 ± 0.1	3.7 ± 0.1	0.9 ± 0.1	1.6 ± 0.1	3.1 ± 0.1	0.6 ± 0.1	3.2 ± 0.1	3.9 ± 0.1	2.8 ± 0.1	5.3 ± 0.2
Cr	1.9 ± 0.7	2.5 ± 0.7	BDL	1.6 ± 0.5	3.2 ± 0.8	2.9 ± 0.5	BDL	5.0 ± 0.9	4.7 ± 1.2	2.0 ± 0.7	2.8 ± 1.1
Mn	31.7 ± 3.1	46.7 ± 2.5	20.9 ± 2.8	54.3 ± 2.4	59.9 ± 4.2	49.1 ± 2.4	38.7 ± 2.1	45.9 ± 4.2	64.4 ± 6.3	52.6 ± 3.8	62.1 ± 5.9
Fe	83.1 ± 3.6	101.8 ± 3.8	106.8 ± 4.1	130.5 ± 2.6	132.5 ± 4.5	180.8 ± 2.9	163.6 ± 2.9	131.7 ± 4.5	384.2 ± 9.8	130.3 ± 4.3	159.8 ± 6.7
Cu	9.3 ± 0.4	11.6 ± 0.4	10.5 ± 0.4	11.1 ± 0.2	12.0 ± 0.4	12.1 ± 0.2	11.7 ± 0.2	12.6 ± 0.4	12.6 ± 0.6	11.3 ± 0.4	11.9 ± 0.6
Zn	4.6 ± 0.7	33.9 ± 1.3	7.4 ± 0.7	6.4 ± 0.4	17.2 ± 1.0	12.2 ± 0.5	6.5 ± 0.4	13.6 ± 1.0	28.2 ± 1.7	13.2 ± 1.0	19.1 ± 1.4
Rb	14.3 ± 1.5	18.7 ± 1.6	34.6 ± 1.9	13.4 ± 1.0	45.9 ± 2.2	25.5 ± 1.1	38.3 ± 1.2	11.9 ± 1.6	34.4 ± 2.7	48.6 ± 2.2	50.8 ± 3.0
Sr	159.3 ± 2.7	92.3 ± 2.2	81.2 ± 2.1	81.2 ± 0.8	48.4 ± 1.8	39.9 ± 1.0	12.7 ± 0.7	43.4 ± 1.8	106.8 ± 3.3	32.9 ± 1.6	73.7 ± 2.7
Pb	19.1 ± 2.5	11.2 ± 2.0	13.5 ± 2.2	8.2 ± 1.4	16.3 ± 2.5	11.5 ± 2.5	6.2 ± 1.4	14.6 ± 2.2	15.2 ± 5.3	7.3 ± 2.5	33.7 ± 5.3

* = Concentration are in ppm, otherwise mentioned; BDL = below detection limit.

diabetes contains high amount of potassium. *Curry* leaves, *Gudmari* leaves and *Swarna champa* leaves contain high concentration of zinc, chromium and rubidium respectively. It was also found that *Swarna champa* leaves contains higher amount of copper. The element strontium was found to be of maximum concentration in *Bella* leaves. However, it can be observed from the Table 2 that the trace elements present in these medicinal plant leaves show a significant variation in their concentration. Some of these plants are found to be rich in one or more individual elements, which pertain their therapeutic value for treatment of different diseases delicately, either singly or in combination. It may also be understood from the above discussions that the elements K, Ca, Cr, Mn, Fe, Cu, Zn are found to play significant role in management and control of diabetes, and they may be in the bio-available form in plants. The strontium and rubidium have no such functions reported till now though both elements are present in these leaves and need to be subject of detailed investigation. The further extensive investigation on the relationship of organic content and trace elements are under progress, and will be reported with better stastics in near future.

Acknowledgments

Heartfelt thaks are due to Mr. P K Rana of Department of Chemistry, Utkal University, Vani Vihar, Bhubaneswar for his active participation in sample preparation.

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